Hydrogen Peroxide Assay Kit

User Manual

Catalog # MBS822356

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Detection and Quantification of Hydrogen Peroxide Concentrations in Urine, Serum, Plasma, Other biological fluids, Tissue Extracts, Cell Lysate, Cell culture media Samples.

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I. INTRODUCTION

Hydrogen Peroxide (H_2O_2) is a reactive oxygen metabolic byproduct that serves as a key regulator for a number of oxidative stress-related states. Functioning through NF-kappaB and other factors, hydroperoxide-mediated pathways have been linked to asthma, inflammatory arthritis, atherosclerosis, diabetic vasculopathy, osteoporosis, neuro-degenerative diseases, Down's syndrome and immune system diseases.

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II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate		
Reagent I	Powder x 1	4 °C
Reagent II	15 ml x 2	4 °C
Reagent III	30 ml x 2	4 °C
Technical Manual	1 Manual	

Note:

Reagent I: add 10ml hydrochloric acid to dissolve it absolutely before use, stored at 4°C.

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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 415 nm
2. Acetone 60ml
3. Hydrochloric acid 10ml
4. Pipettor
5. Pipette tips
6. Mortar
7. Ice
8. Centrifuge (for cell lysis)
9. Timer

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IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 500 ul acetone for 2×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10min, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weight ~0.05g tissue, homogenize with 0.5 ml acetone on ice, centrifuged at 8000g 4 °C for 10min, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

Note:

1. Acetone should be precooled before use because of its volatile, grind the sample on ice.
2. Please wear disposable gloves and masks because of the high volatile of reagents in the kit.

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V. ASSAY PROCEDURE

Preheat Reagent I, II and III on 37 °C (only for mammal) or 25 °C (for general organisms) for 10 minutes.

Reagent	Experiment	Control
Sample	all the supernatant	
Acetone		500 ul
Reagent I	50 ul	50 ul
Reagent II	100 ul	100 ul
Centrifuged at 4000 g, 25 °C for 10 minutes, discard the supernatant, leave the precipitation.		
Reagent III	500 ul	500 ul
Mix, stand at room temperature for 5 minutes, take 200 ul into the microplate, record absorbance measured at 415 nm., absorbances of experiment and control are denoted as A1 and A2, do the control only once.		

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VI. CALCULATION

1. The regression equation under standard condition: Y = 0.062X - 0.08

(X = concentration of standard ; Y = absolute absorbance, A1 - A2)

2. Calculation of H_2O_2 in serum or plasma

H_2O_2 (umol/mL) = (A1 - A2 + 0.08) / 0.062 = 16.13 × (A1 - A2 + 0.08)

3. Calculation of H_2O_2 in cell, bacteria and tissue

(1) According to protein concentration of sample

H_2O_2 (umol/mg prot) = (A1 - A2 + 0.08) / 0.062 / Cpr

Cpr: protein concentration, mg/ml. The protein concentration should be detected separately, you can buy BCA protein concentration assay kit.

(2) According to fresh weight of sample

H_2O_2 (umol/g FW) = (A1 - A2 + 0.08) / 0.062 / (W / Vst)

=8.06 × (A1 - A2 + 0.08) / W

W: fresh weight of sample, g;

Vst: total volume of extract, 0.5ml.

(3) According to concentration of cell or bacteria

H_2O_2 (umol/10^4) = (A1 - A2 + 0.08) / 0.062 / (200 / Vst)

=0.04 × (A1 - A2 + 0.08)

200: Quantity of cell or bacteria, 200×10^4

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VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online

VIII. NOTES

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